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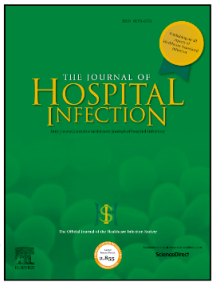

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Specific antibacterial activity of copper alloy touch surfaces in five long-term care facilities for older adults.

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Abstract

Background: Pathogens involved in healthcare-associated infections can quickly spread in the environment, particularly to frequently touched surfaces, which can be reservoirs for pathogens.

Aim: The purpose of this study was to investigate naturally occurring bacterial contamination on touch surfaces in five French long-term care facilities and to compare bacterial populations recovered from copper and control surfaces.

Methods: More than 1300 surfaces were sampled. The collected bacteria were identified to obtain a global view of the cultivable bacterial populations colonizing touch surfaces. Haemolytic colonies and putative pathogens were also screened using specific agar plates and then identified with matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. In total, more than 3400 colonies were analysed.

Findings: *Staphylococcus* and *Micrococcus* were the two predominant genera present on touch surfaces, respectively occurring on 51,8% and 48,0% of control surfaces. In these facilities with relatively low bioburden, copper surfaces efficiently reduced the occurrence frequencies of three genera: *Staphylococcus*, *Streptococcus* and *Roseomonas*. Pathogenic species such as *Staphylococcus aureus*, *Enterococcus faecalis* and *Enterococcus faecium* were observed in very few samples. In addition, methicillin-resistant *Staphylococcus aureus* was observed on five control surfaces and one copper surface.

Conclusion: Contamination of healthcare facilities touch surfaces can be the source for the spread of bacteria through the institution. This in situ study shows that the frequency of the contamination as well as the specific bacterial population bioburden is reduced on copper alloy surfaces.

Keywords

Copper; Antimicrobial surfaces; Healthcare-associated infections; Long-term care facilities; Bacterial identification; MALDI-TOF.

Introduction

Among the risk factors for patients or residents in healthcare facilities, direct skin contact between two people is the main vector for microbial dissemination. Ensuring good hand hygiene is therefore essential. Inanimate environments are also reported as pathogen reservoirs and sources of cross-contamination. In healthcare environments, touch surfaces are one of the main points of cross-contamination, and their involvement in pathogens' spread has already been demonstrated [1]. Numerous studies have shown that bacteria (*e.g. Escherichia coli* [2-4], *Enterococcus faecalis* [5], *Staphylococcus aureus* [5], *Klebsiella pneumoniae* [3]), fungi (*e.g. Candida albicans* [6]) and viruses (*e.g.* adenovirus [7,8], norovirus [9,10]) can survive for several months on dry inanimate surfaces [1,11].

Metals, especially copper, have been drawing continual interest as natural antimicrobial agents. During direct contact between bacteria and a copper containing surface, copper ions are massively released from the surface, at first inducing damages to the cell wall [12,13]. Inside the bacterial cytoplasm, copper ions trigger the formation of reactive oxygen species through the Fenton-type reaction and compete with various metal ions like iron (in iron-sulphur clusters) and zinc for important binding sites on proteins [14-16]. Combined together, these various mechanisms result in rapid bacterial killing. Efficiencies of pure copper and copper alloy surfaces against a wide range of microorganisms have recently been demonstrated in *in vitro* tests [17-33]. For example, Mehtar et al. [21] demonstrated a 5 log reduction of *C. albicans* and 8 log reduction of *K. pneumoniae* within 60 min, a 7 log reduction of *Acinetobacter baumannii* within 180 min, and an 8 log reduction of MRSA and *Pseudomonas aeruginosa* within 270 min.

In situ studies have looked for specific types of pathogens on copper and control touch surface in healthcare facilities [34-37], while another study has initiated a large screening of environmental bacteria present on high-touch athletic center surfaces (copper and control) [38]. However, environmental bacterial populations present in long-term care facilities on copper touch surfaces, have never been characterized before. Previously, we showed that copper touch surfaces reduce overall bacterial contaminations in five long-term care facilities [17]. The aim of this current study was to analyse the diversity of the environmental bacterial populations recovered from copper and control surfaces during 18 months and the impact of copper surfaces on these populations.

Material and methods

Long-term care environment

Five long-term care facilities located in Marne (France) had been 50% outfitted with copper alloy door handles (residents' room) and handrails (corridors) (Steriall®, Lebronze Alloys, Suippes, France) at least 18 months before the start of this study. Depending of the structure of each facility, the design was not the same but after random selection of half of the areas, the outfitted rooms were always close to each other and represented blocks (distinct aisle, distinct levels...). Healthcare workers were requested not to change anything in the surfaces cleaning protocols and to treat all the surfaces the same way. Half of the original residents' room door handles and corridors' handrails were replaced by copper supplies, while the other half remained unchanged and used as control surfaces. The rooms' door handles contained around 90% copper and the handrails around 70% copper. Further information about the long-term care facilities are detailed in Table I.

Study design

Briefly, seven series of sampling were performed in each establishment with at least six days between each series (Figure 1). The sampling series were conducted from June 2016 to December 2017. Rooms were randomly selected before each sampling series and these rooms were excluded from the next series (except in facility *C* which had only 24 rooms). Each series consists of forty samples (copper / control; door handles / handrails), corresponding to 10 copper outfitted rooms, the adjacent copper corridor handrail, and 10 control rooms and their adjacent corridor handrails. All the tested door handles were on corridor side. For each establishment, first and last series were separated by at least 13 months (except for establishment *D*, for which it was only seven months). The last series occurred at least 36 months after copper installation.

Sampling protocol

Samplings were performed on Monday or Tuesday morning, between 7:30 and 9:00, before daily cleaning of surfaces. For each surface tested (door handle or handrail), a moistened cotton swab (Copan, Murrieta, CA, USA) was applied on an area of 10 cm^2 using silicon templates. The area of 10 cm^2 corresponded to the upper surface of the handles or handrails. For handrails, the selected area was systematically sampled at 10 cm from the beginning of the handrail located next to the selected room and going towards the general common rooms. The swabs were then placed in 15 mL tubes containing one mL peptone water (as resuspension medium) and transported to the laboratory at a controlled temperature (4°C). The same person was in charge of all the samplings in the five long-term care facilities and of the sample preparation for the bacterial analysis.

Multi-screening bacterial culture and identification

Less than two hours after sampling, the samples were placed in an ultrasonic bath at 35 kHz for two minutes, then vortexed for thirty seconds to allow the bacteria to resuspend in the medium. Fractions of each sample were inoculated onto various microbiological culture agar plates. Trypticase soy agar plates (TSA) (Biokar Diagnostics, Allonne, France) were used to estimate total number aerobic bacteria. More specific agar plates were used to select specific populations. Haemolytic colonies were screened on TSA + 5% sheep blood agar (BIO-RAD, Hercules, United-States), Enterobacteriaceae family and *Enterococcus* spp on urinary pathogens chromogenic agar (Uri*Select*4™, BIO-RAD, Hercules, United-States) and *S. aureus* on *S. aureus* chromogenic agar (Sa*Select*™, BIO-RAD, Hercules, United-States, or chromID™ *S. aureus*, Biomérieux, Marcy-l'Etoile, France).

Plates were aerobically incubated at 37°C. The total number of colony-forming units (CFU), as well as the number of haemolytic colonies and colonies typically stained on chromogenic agar, were examined and counted at 24h, 48h and 72h. For each type of plate, the method gave a lower detection limit of one CFU/cm^2.

On TSA plates, a maximum of three colonies of each colony phenotype were selected for identification. All the colonies presenting haemolysis on blood agar plates and presenting colour on chromogenic agar were selected for identification. The identification was performed using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry system (IVD MALDI Biotyper, Bruker Daltonique, Wissembourg, France) and the 2017 MTB Compass IVD/RUO data bank following the manufacturer's guidelines.

S. aureus and Enterococcus spp isolates antimicrobial susceptibility testing

Antibiotic susceptibility was determined using the Vitek 2 automated system (Biomérieux, Marcy-l'Etoile, France), according to the manufacturer's guidelines. The interpretation was made using CA-SFM-EUCAST guidelines [39]. MRSA, and Vancomycin Resistant *Enterococcus* (VRE) were defined based on the results of Vitek 2.

Statistical analysis

Qualitative variables were analysed with the two-sided Fisher's exact test or the X^2 test, depending on expected values, using Prism software (version 5, GraphPad Software, San Diego, United-States). The results were considered statistically significant when $P < 0.05$.

Results

Bacterial population diversity recovered from the long-term care facilities

The different types of colonies grown on TSA plate were identified for each 23 sampling series, representing 429 control surfaces and 428 copper surfaces. A total of 2 407 colonies (637 from control and 452 from copper door handles, 745 from control and 573 from copper handrails) were analysed by MALDI-TOF. Successfully identified colonies (62%) belonged to one of the three major clades and were divided into 34 distinct genera: Protobacteria (12 genera), Actinobacteria (12 genera) and Firmicutes (10 genera). At least 76 different species could thus be determined although most of them had few colonies isolated on agar (Table II).

Looking more specifically within each clade (Figure 2), touch surfaces were frequently contaminated by two predominant bacterial genera: *Staphylococcus* spp were observed on 51.8% of control surfaces and 31.1% of copper surfaces, while *Micrococcus* spp, represented only by the species *Micrococcus luteus,* was observed on 48.0% of the control surfaces and 42.8% of the copper surfaces. The other genera were all observed on less than 10% of the surfaces. Among these genera, *Moraxella* spp, *Corynebacterium* spp and *Kocuria* spp were the most commonly found.

As shown in Figure 3, significant differences in the frequency of bacterial carriage as well as in the bacterial burden on surfaces, were found for three bacterial genera that were less frequently detected on copper touch surfaces than on control surfaces. *Staphylococcus* spp were found half as often on copper than on control. *Streptococcus* spp was found fourteen times on control and only one time on copper surface (3.0% control surfaces, 0.34% copper surfaces) and again the number of bacteria collected was much lower. Similarly, *Roseomonas* spp, was found eight times and only on controls (1.9% control surfaces, 0.00% copper surfaces).

Identification of haemolytic colonies

Bacterial haemolytic phenotype is somehow linked to its virulence. Therefore, identification of haemolytic colonies was established for each sample representing a total of 688 samplings on control surfaces and 688 samplings on copper surfaces (Table III). Once again, *Staphylococcus* spp was the most frequent isolated genus. Presenting essentially beta-haemolysis, *Staphylococcus* spp was significantly less frequent on copper surfaces, contaminating 94 copper surfaces and 203 control surfaces. The most frequent genus presenting only alpha-haemolysis was *Streptococcus* spp. Again, it was significantly less frequent on copper surfaces, with its presence occurring on eight control surfaces but never on copper surfaces. Considering all haemolytic bacteria, each identified genus was observed less frequently on copper surfaces, with the exception of *Pseudomonas* spp which was only observed once on copper surface. To note, *Pseudomonas* spp was collected only three times on copper and one time on control surfaces through the identification of the colonies on TSA.

Bacterial species frequently involved in healthcare associated infections (HAI)

Among all the isolates of *Staphylococcus*, 14 species were recovered, representing different levels of contamination. As seen in Figure 4, *S. epidermidis, S. hominis, S. capitis* were the most frequently present on all four surfaces. *S. aureus* was found in high concentration (>10 CFU/cm²) only on the control door handles. *S. pettenkoferi* was quite often recovered but in lower concentrations than the others. For most of the species, copper surfaces were less contaminated than control surfaces. Using *S. aureus* specific chromogenic agar, the rare presence of *S. aureus* was confirmed on 9 out of 652 control surfaces and on 3 out of 653 copper surfaces.

On the chromogenic agar for the isolation of urinary tract pathogens, the presence of *Enterococcus faecalis* was only observed on one control surface, and *Enterococcus faecium* on two control surfaces and one copper surface. With respect to Enterobacteriaceae, the only representative species found were *Escherichia vulneris*, *Pantoea agglomerans* and *Pantoea septica* and each time on only one control surface and never on copper surface.

Resistance profiles of species frequently involved in HAI

To test whether resistant bacteria could survive more specifically on one type of surface, antibiotic susceptibility was determined for colonies of *S. aureus* and *Enterococcus* spp isolated from TSA and chromogenic agar (Table IV). Six of the fourteen isolated strains of *S. aureus* (eleven control strains and three copper surface strains) were MRSA. Five were isolated from the control surface and one from the copper surface. MRSA was collected in four of five long-term care facilities. None of the six *Enterococcus* spp isolates were resistant to vancomycin.

Discussion

Increasing numbers of elderly and immunocompromised people live in long-term care facilities (LTFC) and are particularly vulnerable to infections by pathogens or opportunistic pathogens (skin, urinary tract, respiratory tract infections, pneumonia or gastroenteritis...) [40]. Furthermore, even if a slight decrease in the rate of HAI is observed in long-term care facilities like in hospitals, their threat remains worrying and the prevalence of infected resident in France varies between 3% and 5% with a higher relative frequency of skin and soft tissue infections (20.4%) than in hospitals [41]. *Staphylococcus* spp, mainly coagulase-negative, and *Streptococcus* spp both represent a health concern, as they are among the opportunistic pathogens regularly involved in HAI [41-43]. *S. aureus* represents the leading cause of skin and soft tissue infections in long-term care facilities, with a majority of antibiotic-resistant strains involved [40].

We previously analysed the levels of bacterial burden recovered from door handles and handrails in five long-term care facilities and results have shown a reduction of the number of bacteria found on copper surfaces [17]. Here, in this multicenter study combining one of the largest number of copper fitted rooms and sample collection, we went further and investigated the bacterial populations contaminating these door handles and handrails. We focused on the diversity of the bacterial population recovered and the impact of copper on these populations. More than 3000 colonies isolated on agar, were analysed by MALDI-TOF mass spectrometry. The bacterial populations presented a large diversity with at least 76 different species. *S. aureus*, *E. faecium* and *E. faecalis*, frequent pathogens found in hospitals, sporadically contaminated touch surfaces in theses long-term care facilities. Furthermore, *Staphylococcus* spp, *Streptococcus* spp and *Roseomonas* spp were significantly less frequently observed on copper surfaces. *Micrococcus* spp, *Corynebacterium* spp and *Kocuria* spp belonging to the Actinobacteria phylum, seemed to be less affected by copper surfaces. This was also partially observed in another study where *Micrococcus* spp represented a greater proportion of the colonies on copper touch surfaces [38].

Gram-positive bacteria were largely predominant and environmental microorganisms known to be ubiquitous or typical of the skin flora, such as *Bacillus* spp, *Corynebacterium* spp, *Micrococcus* spp or *Staphylococcus* spp, were regularly isolated from the touch surfaces. *Staphylococcus* spp and *Micrococcus* spp were largely the two most frequent genera found on almost half control surfaces. These results are correlated with the results from studies carried out in hospital [44-47] as well as in an athletic center [38], confirming that Gram-positive bacteria and cutaneous flora bacteria are predominant in the contaminations of touch surfaces.

Enterobacteriaceae were very rare and only three species *Pantoea septica*, *Pantoea agglomerans* and *Escherichia vulneris* were observed. Compared to hospital surfaces [44-47], the contact surfaces of long-term care facilities appeared to be less contaminated by Enterobacteriaceae or even by MRSA. This may be linked to the differences between the two environments but also to the patients' health, sanitary and dressing conditions. In addition, no outbreaks of bacterial gastroenteric infection were reported during the study among the five long-term care facilities. The presence of *Proteus mirabilis*, *K. pneumoniae* or *A. baumannii* was not detected either. *A. lwoffii* and *A. ursingii* were the only *Acinetobacter* spp observed and only on four surfaces. The paucity of these Gram negative bacteria in the samples might have several explanations. Overall, Gram negative bacteria are mostly detected on surfaces very close to patients' bed [45,48] and near water points like sinks [49] but less frequently in environmental surfaces (unlike Gram positive bacteria). Here, we analysed the door handles and handrails surfaces at distance from the residents' bed or water points, which can explain why so few

Enterobacteriaceae were observed. In contrast, we observed that *Moraxella*, a Gram negative bacteria, was regularly present on touch surfaces (9.6% of control surfaces, 7.5% of copper surfaces).

Focusing on bacterial species frequently involved in HAIs [43], *S. aureus*, *Enterococcus* spp, Enterobacteriaceae were rarely observed in this study and no *A. baumannii* could be detected. Among these bacteria, *S. aureus* was the most frequently observed (1.7% of control surfaces and 0.44% of copper surfaces), followed by *Enterococcus* spp (*E. faecium* and *E. faecalis,* 0.58% of control surfaces and 0.29% of copper surfaces). MRSA contamination was sporadic (6 out of 14 strains of *S. aureus* collected) but the level of contamination could be extremely high. In fact, one of the control door handles was contaminated with more than 200 CFU / cm^2 of MRSA, thus representing a high risk of cross-transmission.

Few *Enterococcus* spp were present and no VRE was detected on surfaces through the study. These results are quite different from those obtained in a Greek hospital care unit by Souli et al. (2017) [37]. They observed that *Enterococcus* spp was more frequently present (4.5% of control surfaces and 1.3% of copper surfaces) than *S. aureus* (0.6% of control surfaces and 0.3% of copper surfaces). They also revealed that Gram-negative species frequently involved in HAI were regularly observed on hospital touch surfaces. In particular, they isolated 88 *A. baumannii* (57 on control, 31 on copper surfaces) among 685 samples from touch surfaces. These results correlate with the particularly relative high prevalence of *Acinetobacter* spp in HAI in Greece (16.8%), whereas this prevalence is much lower in France (2.0%) [42].

Our study reports the largest number of samples taken through the longest period in LTCF. While the majority of the bacteria could be identified successfully, about 38% were not identified by MALDI-TOF. This technique is being used routinely in hospitals for the detection of bacteria involved in HAI with reliable results [50,51]. Most of the unidentified strains probably belong to environmental populations.

Furthermore, while the antibacterial effect of copper has been observed against opportunistic pathogens such as coagulase negative staphylococci and streptococci, the frequency of *S. aureus* (eg MRSA was observed in 0.72% of control surfaces and 0.15% of copper surfaces), *Enterococcus* spp and other pathogens involved in HAI was too low to reach a statistical significance difference. However, these results are quite similar to those obtained in a comparable study performed in intensive care units of three hospitals in the United-States, where MRSA were observed on 0.63% of control surfaces and 0.3% of copper surfaces [36]. Thus, the real impact of copper surfaces on MRSA, VRE and other pathogens is difficult to validate statistically in clinical settings [35,36].

The LTFC involved in this study have different elderly populations (bedridden, autonomous, urban, rural…) but no significant differences were observed between them. Nevertheless, the lack of resident-related outcomes and the impossibility to link all the resident epidemiological data to the microbiological results is a limitation to gain insight into the impact of copper on the resident infections. Some studies, conducted in intensive, intermediate and acute care units, outfitted with copper surfaces have investigated this issue and although statistical significance is rarely achieved, the results suggest that there are fewer infections in premises with a copper surface [52-54]. A recent retrospective study [55] involving nursing home (establishment E), has shown that outbreaks caused by viral pathogens transmitted by hand involved significantly less residents from areas equipped with copper (11 cases of infection) than from control areas (44 cases). All these studies suggest that copper equipment may represent a solution to reduce the infections risk in nursing homes as well as in hospitals.

Conclusion

Environmental as well as skin bacterial flora can be as harmful as pathogenic bacteria for the elderly and the immunocompromised. In this context, preventing skin colonization and bacterial spread during contact with touch surfaces is essential. Copper alloys touch surfaces containing at least 70% of copper effectively contribute to limit the survival and spread of microorganisms in healthcare facilities, thus reducing the overall risk of hand transmission of opportunistic pathogenic bacteria. To confirm that copper alloys touch surfaces may present a global health benefits for patients, residents and healthcare workers, additional prospective studies still need to be carried out.

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Conflicts of Interest

The authors declare that the research was partially conducted with support from Le Bronze alloys (convention CIFRE 201411394). The society was in charge of the copper alloys installation in long-term care facilities, but was not involved in the collection, analysis and interpretation of the data or in the decision to publish the results.

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Table I. General information about long-term care facilities and copper installation.

Long-term care facilities (codes)	Number of rooms (Copper /Total)	Number of beds (Copper /Total)	Nature of touch surfaces		Date of copper installation
			Door handles	Handrails	
A	43/82	53/99	PVC	Wood	July 2014
B	54/117	54/117	PVC	PVC	July 2014
C	12/24	12/25	Stainless steel	PVC	October 2014
D	30/56	30/57	Aluminium or PVC	Aluminium or Wood	June 2014
E	158/347	158/347	PVC	Wood	June 2014

Table II. Overview of the bacterial diversity and frequency on touch surfaces. Bacteria were cultured on TSA, identified using MALDI-TOF and classified using a phylogenetic approach (SeaView 4 software, BioNJ method).

		Numbers of positive surfaces	
		Control (total = 429)	Copper (total = 428)
Proteobacteria	*Acinetobacter lwoffii*	3	1
	Acinetobacter ursingii	0	4
	Moraxella sp	34	30
	Pseudomonas oryzihabitans	0	2
	Pseudomonas stutzeri	1	1
	Pantoea septica	1	0
	Massilia timonae	1	3
	Neisseria flavescens	1	0
	Pseudoxanthomonas kaohsiungensis	1	1
	Roseomonas mucosa	8	0
	Brevundimonas vesicularis	1	0
	Agrobacterium tumefaciens	0	1
	Paracoccus yeei	10	12
	Sphingomonas melonis	0	1
	Sphingomonas paucimobilis	1	0
Actinobacteria	*Actinomyces oris*	0	1
	Corynebacterium amycolatum	1	0
	Corynebacterium ammoniagenes	0	1
	Corynebacterium aurimucosum	1	1
	Corynebacterium striatum	1	0
	Corynebacterium lipophiloflavum	0	1
	Corynebacterium coyleae	6	0
	Corynebacterium mucifaciens	2	0
	Corynebacterium tuberculostearicum	17	12
	Dietzia sp	1	2
	Gordonia sp	0	1
	Brevibacterium casei	1	3
	Kytotoccus sedentarius	1	0
	Kocuria palustris	4	2
	Kocuria marina	0	2
	Kocuria rhizophila	8	7
	Arthrobacter sp	0	1
	Micrococcus luteus	205	184
	Microbacterium oxydans	0	1
	Microbacterium paraoxydans	1	1
	Pseudoclavibacter endophyticus	0	1
	Streptomyces violaceoruber	1	0

		Numbers of positive surfaces	
		Control (total = 429)	Copper (total = 428)
Firmicutes	*Brevibacillus centrosporus*	1	0
	Paenibacillus barengoltzii	2	0
	Paenibacillus amycolyticus	0	2
	Paenibacillus urinalis	5	3
	Exiguobacterium sp	1	0
	Enterococcus faecalis	1	0
	Enterococcus facecium	1	1
	Globicatella sp	1	0
	Lactococcus lactis	1	0
	Streptococcus oralis	2	0
	Streptococcus cristatus	1	0
	Streptococcus parasanguinis	1	0
	Streptococcus anginosus	1	0
	Streptococcus salivarius	3	0
	Streptococcus thermophilus	8	1
	Fictibacillus arsenicus	1	1
	Bacillus cereus	5	2
	Bacillus flexus	5	3
	Bacillus megaterium	1	0
	Bacillus indicus	0	1
	Bacillus infantis	0	1
	Bacillus horneckiae	1	0
	Bacillus simplex	0	2
	Bacillus licheniformis	1	3
	Bacillus subtilis	0	1
	Staphylococcus condimenti	1	1
	Staphylococcus simulans	6	7
	Staphylococcus cohnii	6	5
	Staphylococcus pettenkoferi	48	23
	Staphylococcus saprophyticus	1	3
	Staphylococcus aureus	6	2
	Staphylococcus capitis	69	36
	Staphylococcus caprae	0	1
	Staphylococcus epidermidis	93	42
	Staphylococcus pasteuri	7	1
	Staphylococcus warneri	7	6
	Staphylococcus haemolyticus	17	4
	Staphylococcus hominis	62	36
	Staphylococcus lugdunensis	2	0

Table III. Number of surfaces colonized by haemolytic bacteria. For each surface group n= 688.

		Control surfaces Number (%)	Copper surfaces Number (%)	*P* value (Fisher's exact test / X^2 test)
Beta-haemolysis	Unidentified	44 (6.40)	17 (2.48)	-
	Aerococcus spp	1 (0.15)	1 (0.15)	1
	Bacillus spp	5 (0.73)	4 (0.58)	0.7530
	Micrococcus spp	1 (0.15)	0 (0.00)	1
	Staphylococcus spp	203 (29.55)	94 (13.70)	**< 0.0001**
	Staphylococcus aureus	5 (0.73)	2 (0.29)	0.0814
Alpha-haemolysis	Unidentified	4 (0.58)	2 (0.29)	-
	Aerococcus spp	1 (0.15)	0 (0.00)	1
	Micrococcus spp	5 (0.73)	2 (0.29)	0.2876
	Paenibacillus spp	4 (0.58)	3 (0.44)	1
	Paracoccus spp	2 (0.29)	0 (0.00)	0.2495
	Pseudomonas spp	0 (0.00)	1 (0.15)	1
	Staphylococcus spp	2 (0.29)	0 (0.00)	0.4996
	Streptococcus spp	8 (1.16)	0 (0.00)	**0.0038**

Table IV. Number of drug resistant bacteria detected among the total *S. aureus* and *Enterococcus* spp. MRSA vs *S. aureus*, VRE vs *Enterococcus* spp.

Long-term care facilities	*S. aureus* (MRSA/total)		*Enterococcus* (VRE/total)	
	Control	Copper	Control	Copper
A	2/4	1/1	0/2	0/1
B	1/2	0/0	0/1	0/0
C	1/1	0/1	0/0	0/0
D	0/1	0/1	0/1	0/1
E	1/3	0/0	0/0	0/0
TOTAL	**5/11**	**1/3**	**0/4**	**0/2**

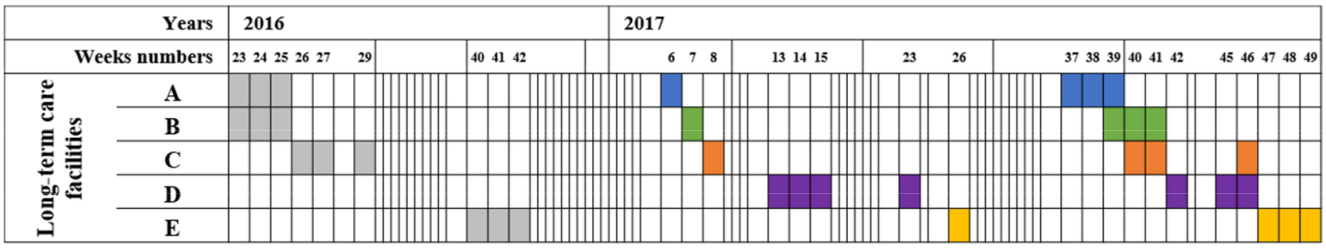

Fig. 1. Organisation of sampling series through the 18 months of the studies. Full boxes represent weeks of sampling series. Coloured boxes represent bacterial identification from TSA, blood and chromogenic agar plates, grey boxes from blood and chromogenic agar plates.

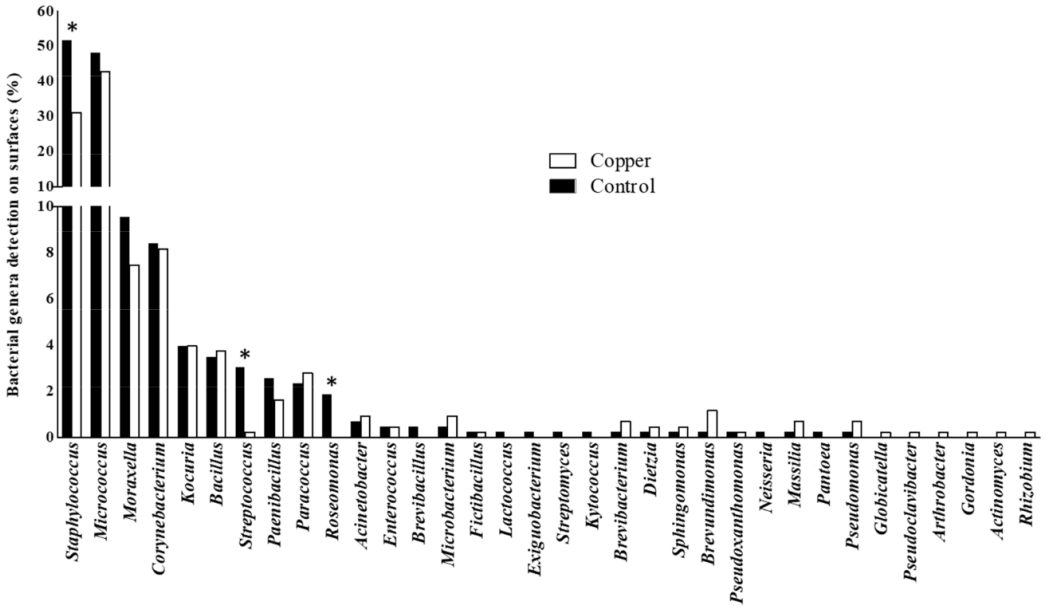

Fig. 2. Percentage of surfaces contaminated by each bacterial genus. * indicates significant difference between the percentage of copper contaminated surfaces and control contaminated surfaces (Fisher's exact test or C^2 test, $P < 0,05$).

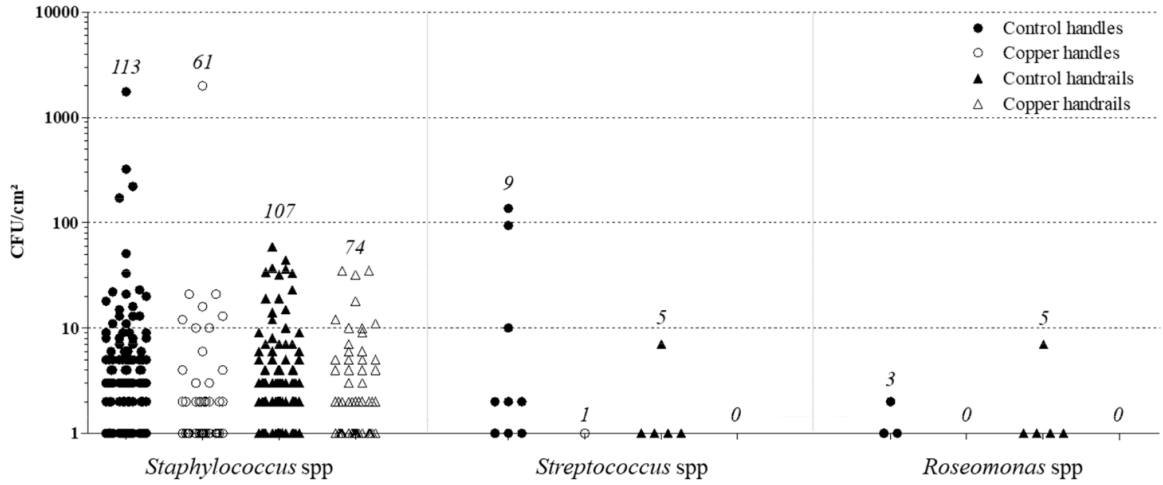

Fig. 3. Touch surfaces contamination levels by the three bacterial genera significantly impacted by copper. Each point represents the bacterial quantity on a single surface. Numbers of contaminated surfaces are noted in italic.

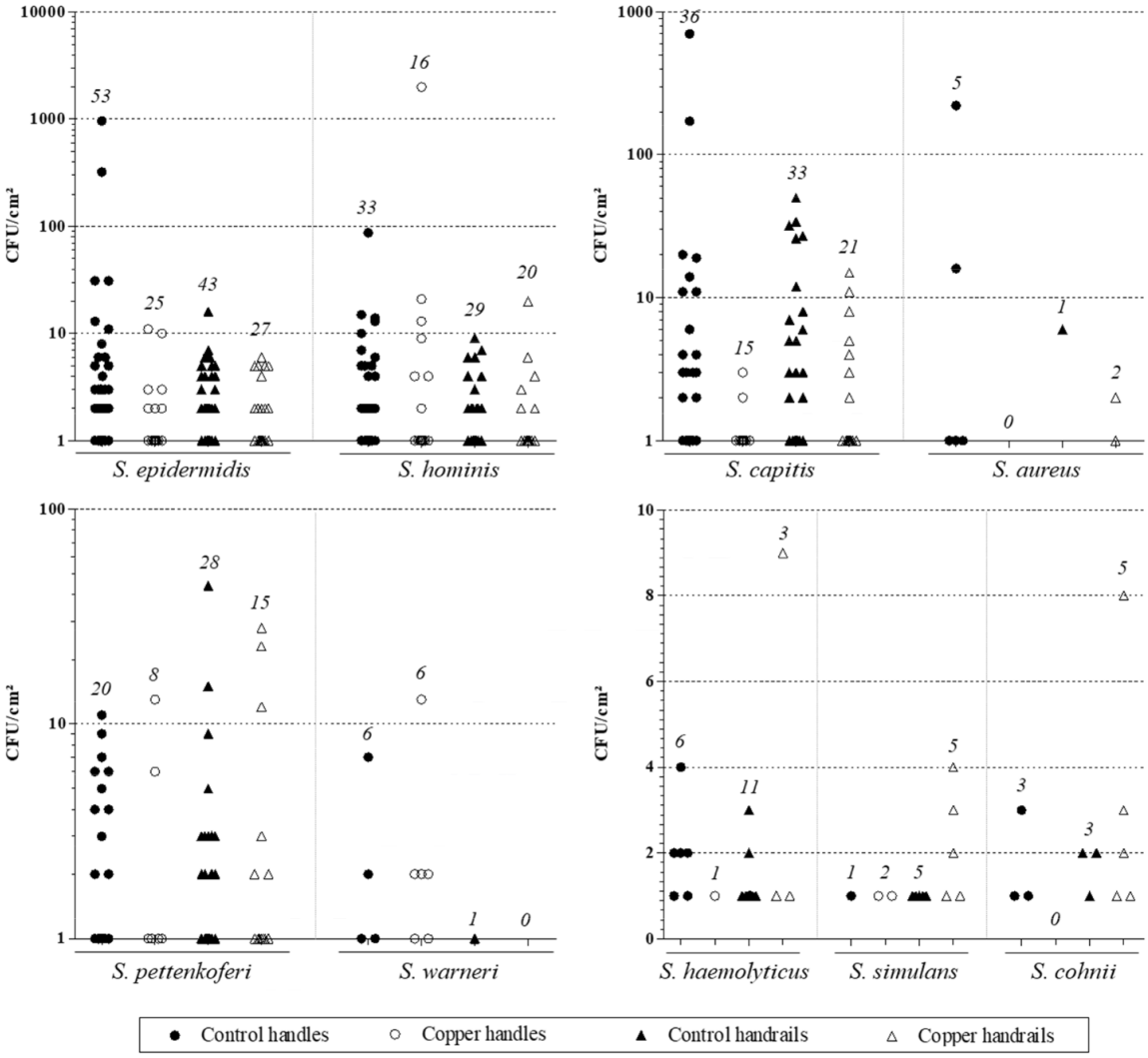

Fig. 4. Bacterial contamination levels of the predominant species of *Staphylococcus* on touch surfaces. Each point represents the bacterial quantity on a single surface. Numbers of contaminated surfaces are noted in italic.

		Numbers of positive surfaces	
		Control (total = 429)	Copper (total = 428)
Proteobacteria	*Acinetobacter lwoffii*	3	1
	Acinetobacter ursingii	0	4
	Moraxella sp	34	30
	Pseudomonas oryzihabitans	0	2
	Pseudomonas stutzeri	1	1
	Pantoea septica	1	0
	Massilia timonae	1	3
	Neisseria flavescens	1	0
	Pseudoxanthomonas kaohsiungensis	1	1
	Roseomonas mucosa	8	0
	Brevundimonas vesicularis	1	0
	Agrobacterium tumefaciens	0	1
	Paracoccus yeei	10	12
	Sphingomonas melonis	0	1
	Sphingomonas paucimobilis	1	0
Actinobacteria	*Actinomyces oris*	0	1
	Corynebacterium amycolatum	1	0
	Corynebacterium ammoniagenes	0	1
	Corynebacterium aurimucosum	1	1
	Corynebacterium striatum	1	0
	Corynebacterium lipophiloflavum	0	1
	Corynebacterium coyleae	6	0
	Corynebacterium mucifaciens	2	0
	Corynebacterium tuberculostearicum	17	12
	Dietzia sp	1	2
	Gordonia sp	0	1
	Brevibacterium casei	1	3
	Kytotoccus sedentarius	1	0
	Kocuria palustris	4	2
	Kocuria marina	0	2
	Kocuria rhizophila	8	7
	Arthrobacter sp	0	1
	Micrococcus luteus	205	184
	Microbacterium oxydans	0	1
	Microbacterium paraoxydans	1	1
	Pseudoclavibacter endophyticus	0	1
	Streptomyces violaceoruber	1	0

		Numbers of positive surfaces	
		Control (total = 429)	Copper (total = 428)
Firmicutes	*Brevibacillus centrosporus*	1	0
	Paenibacillus barengoltzii	2	0
	Paenibacillus amycolyticus	0	2
	Paenibacillus urinalis	5	3
	Exiguobacterium sp	1	0
	Enterococcus faecalis	1	0
	Enterococcus facecium	1	1
	Globicatella sp	1	0
	Lactococcus lactis	1	0
	Streptococcus oralis	2	0
	Streptococcus cristatus	1	0
	Streptococcus parasanguinis	1	0
	Streptococcus anginosus	1	0
	Streptococcus salivarius	3	0
	Streptococcus thermophilus	8	1
	Fictibacillus arsenicus	1	1
	Bacillus cereus	5	2
	Bacillus flexus	5	3
	Bacillus megaterium	1	0
	Bacillus indicus	0	1
	Bacillus infantis	0	1
	Bacillus horneckiae	1	0
	Bacillus simplex	0	2
	Bacillus licheniformis	1	3
	Bacillus subtilis	0	1
	Staphylococcus condimenti	1	1
	Staphylococcus simulans	6	7
	Staphylococcus cohnii	6	5
	Staphylococcus pettenkoferi	48	23
	Staphylococcus saprophyticus	1	3
	Staphylococcus aureus	6	2
	Staphylococcus capitis	69	36
	Staphylococcus caprae	0	1
	Staphylococcus epidermidis	93	42
	Staphylococcus pasteuri	7	1
	Staphylococcus warneri	7	6
	Staphylococcus haemolyticus	17	4
	Staphylococcus hominis	62	36
	Staphylococcus lugdunensis	2	0